South African Journal of Botany 2005, 71(1): 49–52
Printed in South Africa — All rights reserved

SOUTH AFRICAN JOURNAL OF BOTANY
EISSN 1727–9321

Alkaloids from three ethnomedicinal *Haemanthus* species: *H. albiflos*, *H. deformis* and *H. pauculifolius* (Amaryllidaceae)

NR Crouch[1,2]*, TL Pohl[2], DA Mulholland[2] and E Ndlovu[3]

[1] *Ethnobotany Unit, South African National Biodiversity Institute, PO Box 52099, Berea Road 4007, South Africa*
[2] *Natural Products Research Group, Department of Chemistry, University of KwaZulu-Natal, Durban 4041, South Africa*
[3] *Inkomfe Cluster Project, PO Box 504, Estcourt 3310, South Africa*
* *Corresponding author, e-mail: crouch@sanbi.org*

Received 26 April 2004, accepted in revised form 15 June 2004

Three closely-allied ethnomedicinal *Haemanthus* (Amaryllidaceae) species from the summer-rainfall region of South Africa have been phytochemically investigated and have yielded a range of isoquinoline alkaloids. *H. albiflos* yielded homolycorine, albomaculine and the *O*-methyl-lycorenium salt. From *H. pauculifolius*, homolycorine, the novel paucamine present as a salt, and the quaternary salts of homolycorine, montanine and manthidine were isolated. *H. deformis* yielded coccinine, montanine and the quaternary salt of manthidine.

Introduction

The bulbous genus *Haemanthus* L. (Tribe Haemantheae; Amaryllidaceae) comprises 22 species restricted to southern Africa (Snijman 2000), including a closely-related group of three taxa possessing white flowers and an evergreen habit. All three species are shade-loving and occur in the summer rainfall region (Snijman 1984, Snijman and Van Wyk 1993). This distinct set comprises *Haemanthus pauculifolius* Snijman & Van Wyk, *H. albiflos* Jacq. (= *H. albomaculatus* Bak.) and *H. deformis* Hook.f. all of which are traded in the Zulu ethnomedicinal markets of Durban, South Africa (Cunningham 1988, Pettit 1998), the last two as *uzaneke*. In the Eastern Cape the Xhosa drink bulb infusions of *H. albiflos* to promote the healing of broken bones and apply the roots of *H. albiflos* as a poultice over fractures for the same purpose (Dold and Cocks 2000). The Xhosa also use bulb infusions of *H. albiflos* to treat chronic coughs (Broster 1982). Extracts of bulbs of this species have been shown to possess strong antiviral activity (Husson *et al*. 1993) and cytotoxicity (Viladomat *et al*. 1997), but are reportedly not antibacterial (Hutchings *et al*. 1996). Amenorrhea and pregnancy-related problems amongst the Zulu are treated with a decocotion of either *H. deformis* or *H. albiflos* bulbs prepared with bulbous material of two other amaryllids, *Scadoxus puniceus* (L.) Friis & Nordal and *Crinum macowanii* Bak. The medication is taken orally or as an enema. Such a traditional sexual health application may relate to the Doctrine of Signatures principle, given that sterile *H. deformis* plants resemble the human female pubic region. This species is known to the Zulu as the 'female' *uzaneke* (Crouch *et al*. 2002). The purpose of this investigation was to determine the constituents and uses of three Zulu *Haemanthus* species as part of an ongoing ethnobotanical and phytochemical survey of the southern African Amaryllidaceae.

Materials and Methods

Plant materials

Fresh bulbs (2.005kg) and leaves (1.043kg) of *Haemanthus albiflos* Jacq. were collected from Zululand and a voucher (*Crouch 804*, NH) lodged for verification purposes. Fresh bulbs (950g) of *Haemanthus deformis* Hook.f. were obtained from the Warwick Triangle ethnomedicinal market in Durban (*Crouch 820*, NH), and fresh *Haemanthus pauculifolius* Snijman & Van Wyk bulbs (2.624kg) and leaves (617.25g) harvested from the Paris Dam site in Vryheid District, KwaZulu-Natal (*Symmonds 3*, NH).

Extraction and fractionation

The bulbs and leaves of *H. albiflos* were macerated and extracted separately in 2l ethanol at room temperature with continuous agitation for approximately four days. The gum-like residue obtained in both cases was partitioned in 50:50 dichloromethane:methanol solvent. Column chromatography over silica gel (Merck 9385) was then employed to effect the separation of the compounds using various ratios of dichloromethane:methanol.

The bulbs and leaves of *H. pauculifolius* were macerated and extracted separately in 2l ethanol at room temperature with continuous agitation for approximately two and four

days respectively. The gum-like residue was chromatographically separated in a manner identical to that employed for *H. albiflos*.

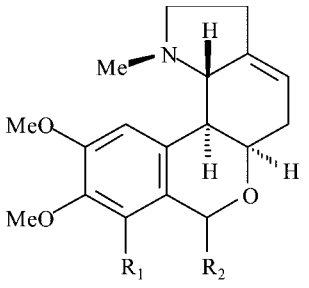
The bulbs of *H. deformis* were macerated and extracted in 1l ethanol at room temperature with continuous agitation for approximately three days. The gum-like residue obtained was partitioned and the component alkaloids separated as for *H. albiflos*.

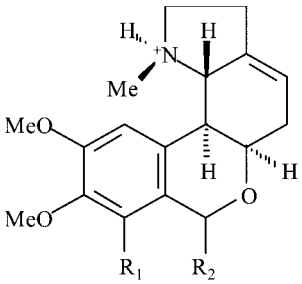
Structure determinations

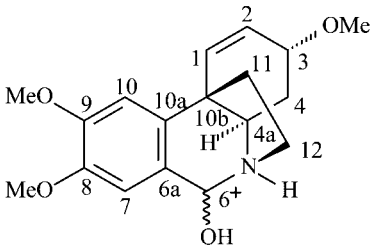
IR spectra were recorded with a Nicolet Impact 400 D spectrometer on sodium chloride plates and calibrated against an air background. EIMS were obtained using a Finnigan 1020 spectrophotometer operating at 70eV. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 400 MHz spectrometer. CD spectra were recorded using a JASCO J700 spectro-polarimeter. The structures of compounds **1** (Bastida *et al.* 1987, Jeffs *et al.* 1985), **2** (Bastida *et al.* 1987), **3** (Codina *et al.* 1993), **5** (Ishizaki and Hoshino 1992, Wildman and Brown 1968), **6** and **7** (Ishizaki and Hoshino 1992) were determined using NMR spectroscopy and mass spectrometry and subsequent comparison against literature values and, in the case of **5, 6** and **7**, the stereochemistry was confirmed by use of CD spectroscopy (Wagner *et al.* 1996). The quaternary salts **3a**, **5a** and **6a** were converted into the free bases **3**, **5** and **6** by treatment with NaOH. The elucidation of the tentative structure of **4** is discussed below.

Results and Discussion

A total of seven alkaloids of the isoquinoline type were isolated (Figure 1), present either as free bases or as quaternary salts. The investigation of both bulbs and leaves of *H. albiflos* yielded homolycorine (**1**), albomaculine (**2**) and the *O*-methyl-lycorenium salt (**3a**). Homolycorine (**1**) was also isolated from *H. pauculifolius* as well as four additional



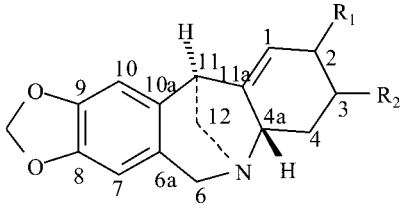


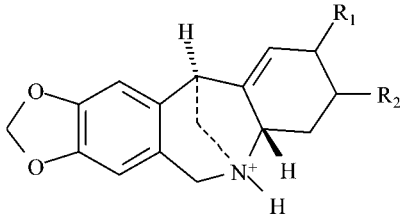


4. Paucamine salt

	R_1	R_2
1. Homolycorine	H	=O
2. Albomaculine	OMe	=O
3. *O*-methyl lycorenine	H	OMe

	R_1	R_2
1a. Homolycorinium salt	H	=O
3a. *O*-Methyl-lycorenium salt	H	OMe





	R_1	R_2
5. Montanine	α-OMe	β-OH
6. Manthidine	α-OMe	α-OH
7. Coccinine	β-OMe	β-OH

	R_1	R_2
5a. Montanine salt	α-OMe	β-OH
6a. Manthidine salt	α-OMe	α-OH

Figure 1: Alkaloids isolated from *Haemanthus albiflos*, *H. deformis* and *H. pauculifolius*

alkaloids, the novel paucamine (**4**) present as a salt, and the quaternary salts of homolycorine (**1a**), montanine (**5a**) and manthidine (**6a**). All compounds were found present in both bulbs and leaves. The investigation of bulbs of *H. deformis* produced three alkaloids: coccinine (**7**), montanine (**5**) and the quaternary salt of manthidine (**6a**). The structure of paucamine was determined, using NMR and CD data, to be a novel crinane (5,10b-ethanophenanthridine) alkaloid. The compound was isolated as the quaternary salt (Figure 1). The ^{1}H NMR spectrum revealed a 'doubling up' of various resonances, typical of a C-6 epimeric mixture where the two C-6 isomers are interconvertible and hence inseparable by chromatographic methods (Ali *et al.* 1981, King *et al*. 1965), indicating an oxygenated substituent at C-6. The absence of the typical AB pattern for the methylene protons at about 4ppm, which is usually observed for crinane alkaloids, further confirmed this. The resonances of one epimer are dominant and are used here to describe the structural elucidation of the compound. The substitution pattern on ring A was confirmed by the presence of two singlets in the aromatic region of the ^{1}H NMR spectrum at δ7.10 (H-10) and δ7.01 (H-7), which were observed in conjunction with two methoxy group proton singlet resonances at δ3.88 and δ3.92. The resonance at δ3.88 showed a positive NOESY correlation to H-7, thus it was placed at C-8. Similarly, the NOESY correlation between H-10 and the resonance at δ3.92 indicated the second methoxy group was present at C-9. Doublet and double-doublet resonances at δ6.81 (*J* = 10.3) and δ6.19 (*J* = 5.3, 10.2) are typical of the H-1 and H-2 alkene protons and served as a confirmation of the *trans* relationship between the C-3 substituent and the 5,10b-ethano bridge. The ABX splitting of H-2 is indicative of a *trans* relationship as opposed to a *cis* relationship where the H-2 proton is typically split into a doublet (Haugwitz *et al.* 1965). The presence of an aliphatic methoxy group proton resonance at δ3.42, which showed a NOESY correlation with the H-2 resonance, indicated the presence of a methoxy group at C-3. Additional resonances typical of compounds of this type are the pair of triplets of doublets at δ1.98 (*J* = 4.2, 9.3, 13.6) and δ2.28 (*J* = 4.4, 9.0, 13.4) which are typical of the H-4 protons, the H-4a double doublet δ4.15 (*J* = 4.3, 13.4) and the H-6 proton singlet at δ5.74, which corresponds to a carbon resonance at δ89.45 (C-6). The H-11 and H-12 resonances were difficult to observe in the ^{1}H NMR spectrum and appeared shortened and broadened. However, their presence could be confirmed using the HSQC spectrum. The two H-11 resonances were observed as multiplets at δ2.19 and δ2.30 and correlated with a methylene carbon resonance at δ39.27, and the H-12 resonances at δ3.85 and δ3.30 correlated with a methylene carbon resonance at δ49.01. The H-6, H-4a and H-12 protons were observed at shifts relatively downfield of their expected positions indicating the presence of a quaternary salt. The mass spectrum gave a parent ion peak at *m/z* 317, consistent with the required $C_{18}H_{23}NO_4$ (free base). It was initially thought that compound **4** was papyramine which has an α-5,10b-ethano bridge. However, when circular dichroism spectroscopy was used to determine the absolute configuration of the 5,10b-ethano bridge**,** it was found that positive Cotton effects were observed at 285nm, 243nm and 226nm. This indicated a β-configuration for the bridge. An α-configuration for the 5,10b-ethano bridge results in negative Cotton effect at 243nm (Viladomat *et al.* 1996, Wagner *et al.* 1996). Thus compound **4** was named paucamine. On treatment with base this compound did not produce the free base but rather predictably decomposed, due to presence of a hemiacetal ring in the molecule. Accordingly, a high resolution mass spectrum could not be obtained.

Paucamine salt**:** (12mg), amorphous pale orange solid, ($C_{18}H_{24}NO_4$), EIMS: 317 (M^+ of free base), 285 (M^+ of free base-CH_3OH), 241. IR: ν_{max}(NaCl)cm^{-1} : 3358, 2935, 1516. $[\alpha]_D$ = +35° [c = 0.005g ml^{-1}, MeOH]. CD: λ_{max} : +226, +243, +285nm. NMR data are given in Table 1.

Traditionally, Amaryllidaceae plant materials are subjected to an acid/base extraction where the aqueous extract of the fresh plant material is sequentially treated with acid and then base and extracted into chloroform. In the case of the three *Haemanthus* species investigated in this work, the general acid/base procedure was not followed as it has been found that this procedure can lead to the non-detection of other interesting compounds (Koorbanally *et al.* 2000). If the acid/base extraction procedure had been followed, the isolation of the salts would not have occurred, as base treatment results in the formation of free base alkaloids.

Acknowledgements — The Mazda Wildlife Fund has supported the Ethnobotany Programme of the National Botanical Institute. The Department of Environmental Affairs and Tourism (DEAT) has generously funded MEDBASE, the National Medicinal Plants Database for South Africa. Mr Richard Symmonds kindly helped collect plant material of *H. pauculifolius*. This research was funded by the National Research Foundation (NRF) and the University of Natal Research Fund. We gratefully acknowledge the Welcome Trust Equipment grant number 052451 for the provision of the 400 MHz NMR spectrometer. We are grateful to Mr Dilip Jagjivan and Mr Bret Parel for running NMR and mass spectra. The staff of the Mary Gunn Library (PRE) are thanked for facilitating access to literature.

References

Ali AA, Kating H, Frahm AW (1981) Four 6-hydroxylated alkaloids in the crinine series from *Crinum augustum.* Phytochemistry **20**: 1731–1733

Bastida J, Vildomat F, Llabres JM, Codina C, Feliz M, Rubiralta M (1987) Alkaloids from *Narcissus confusus.* Phytochemistry **26**: 1519–1524

Broster JA (1982) Amagquira. Religion, Magic and Medicine in Transkei. Via Afrika Limited, Cape Town, 126pp. ISBN 0 7994 0640 6

Codina C, Bastida J, Viladomat F, Fernandez JM, Bergonon S, Rubiralta M, Quirion J (1993) Alkaloids from *Narcissus munozii-garmendiae*. Phytochemistry **32**: 1354–1356

Crouch NR, Chetty J, Mulholland DA, Ndlovu E (2002) Bulb alkaloids of the reputedly psychoactive *Brunsvigia radulosa* (Amaryllidaceae). South African Journal of Botany **68**: 86–89

Cunningham AB (1988) An investigation of the herbal medicine trade in Natal/KwaZulu. INR Investigational Report 29. University of Natal, Pietermaritzburg, p 149

Dold T, Cocks M (2000) Indigenous plant use of the amaXhosa people on the eastern border of the Great Fish River Reserve, Eastern Cape. Annals of the Eastern Cape Museums **1**: 26–53

Table 1: NMR data for paucamine, **4** (400MHz, CD_3OD). Data for minor epimer are given in square brackets

	^1H	^{13}C	HMBC C→H	NOESY
1	6.81(d 10.3) [6.76 (d 10.3)]	130.34 [130.25]	–	2, 10
2	6.19 (dd 5.3, 10.2)	127.63 [127.59]	–	1,3,OMe at C-3
3	4.01m	72.34 [72.06]	1,OMe at C-3	2,4α,β,OMe at C-3
4α	1.98 (ddd 4.2,9.3,13.6)	27.55 [39.96]	2	–
4β	2.28 (ddd 4.4,9.0,13.4)			
4a	4.15 (dd 4.3,13.4)	59.41 [65.31]	1,4,6	4a
6	5.74s [6.35s]	89.45 [88.13]	7,12b	7,12b
6a	–	122.96 [123.72]	6,10	–
7	7.01s [7.06s]	113.57 [112.38]	6	6,OMe at C-8
8	–	150.14	7,10,OMe at C-8	–
9	–	151.69	7,10,OMe at C-9	–
10	7.10s	107.14 [107.20]	–	1,OMe at C-9
10a	–	135.47 [135.25]	1,6,7,11	–
10b	–	46.08 [46.31]	1,2,10,11	–
11a	2.19m	39.27 [39.96]	–	11b
11b	2.30m			11a
12a	3.85m [3.95m]	49.01 [43.55]	–	12b
12b	3.30m [3.62m]			12a
OMe (C-3)	3.42s	56.91 [57.00]	4a	2,3
OMe (C-8)	3.88s [3.87s]	56.59 [56.57]	–	7
OMe (C-9)	3.92s [3.91s]	56.67 [56.70]	–	10

Haugwitz RD, Jeffs PW, Wenkert E (1965) Proton magnetic resonance spectral studies of some Amaryllidaceae Alkaloids of the 5, 10b-ethanophenanthridine series and of criwelline and tazettine. Journal of the Chemical Society 2001–2009

Husson GP, Vilagines P, Sarrette B, Vilagines R (1993) Investigations on antiviral action of *Haemanthus albiflos* natural extract. Phytotherapy Research **7**: 348–351

Hutchings A, Scott AH, Lewis G, Cunningham A (1996) Zulu Medicinal Plants. An Inventory. University of Natal Press, Pietermaritzburg, 450pp. ISBN 0 86980 923 7

Ishizaki MM, Hoshino O (1992) Total synthesis of montanine-type Amaryllidaceae alkaloids which possess a 5,11-methanomorphanthridine ring system, through cyclisation with sodium bis (2-methoxyethoxy) aluminium hydride: the first stereoselective synthesis of montanine, coccinine, O-acetylmontanine, pancracine and brunsvigine. Journal of Organic Chemistry **57**: 7285–7295

Jeffs PW, Abou-Donia A, Campau D, Staiger D (1985) Structure of 9-O-demethylhomolycorineand 5α-homolycorine. Alkaloids of *Crinum defixum*, *C. scabrum*, and *C. latifolium*. Assignment of aromatic substitution patterns from ^1H and ^{13}C spectra. Journal of Organic Chemistry **50**: 1732–1737

King RW, Murphy CF, Wildman WC (1965) 6-Hydroxycrinamine and haemanthidine. Journal of the American Chemical Society **87**: 4912–4917

Koorbanally N, Mulholland DA, Crouch NR (2000) Alkaloids and triterpenoids from *Ammocharis coranica* (Amaryllidaceae). Phytochemistry **54**: 93–97

Pettit G (1998) Traditional medicine. Herbertia **53**: 160–163

Snijman D (1984) A revision of the genus *Haemanthus* L. (Amaryllidaceae). Journal of South African Botany, Supplementary Vol. 12, p 139. ISBN 0 620 07339

Snijman DA (2000) Amaryllidaceae. In: Leistner OA (ed) Seed Plants of Southern Africa: Families and Genera. Strelitzia **10**. National Botanical Institute, Pretoria, pp 570–576. ISBN 1–919795–51–0

Snijman DA, Van Wyk AE (1993) A new species of *Haemanthus* (Amaryllidaceae) from the eastern Transvaal Escarpment, South Africa. South African Journal of Botany **59**: 247–250

Viladomat F, Almanza GR, Bastida J, Campbell WE, Mathee S (1996) Alkaloids from *Brunsvigia orientalis.* Phytochemistry **43**: 1379–1384

Viladomat F, Bastida J, Codina C, Nair JJ, Campbell WE (1997) Alkaloids of the South African Amaryllidaceae. In: Pandalai SG (ed) Recent Research Developments in Phytochemistry. Vol. 1. Research Signpost, Trivandrum, pp 131–165. ISBN 81–86481–08–7

Wagner J, Pham HL, Dopke W (1996) Alkaloids from *Hippeastrum equestre.* Circular dichroism studies. Tetrahedron **52**: 6591–6600

Wildman WC, Brown CL (1968) Mass spectra of 5,11b-methanomorphanthradine alkaloids. The structure of panacrine. Journal of the American Chemical Society **90**: 6439–6446

Edited by J van Staden